

Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps

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Electronic Supplementary Material-2: Behavioural Data Collection.

Focal pupae intended for adult-stage behavioural observations received a paint-mark on their silken cocoon to designate them as “do not collect,” and were allowed to develop and emerge as adults. Those that successfully emerged were marked with distinguishing paint marks and their behaviour recorded for 5-10 days. Behaviours were recorded once a day *via* scan sampling. In 2013, behaviour scans were conducted before 10 00. In 2014, behaviour scans were conducted in the afternoon, after all colonies were allowed to forage freely. In both years, a scan consisted of six observations per individual every 15 minutes. We recorded whether the focal female was on her nest, and if so, which behaviour she was performing (inactive, tending or checking brood, interacting with nestmates, or constructing nest cells). Individuals that were not observed on or near the nest by the 5th day of observations, and were not observed on or near the nest during subsequent nest checks, were recorded as having ‘abandoned’ the nest.

Only a fraction of the pre-pupae marked for behavioural analyses survived to adulthood. Due to low sample size of emerging adults, and the fact that nutrition was restricted only in 2014, we separated our analyses by year. We tested the effects of artificial drumming using only adults from 2013. In 2014, only one wasp emerged from either drumming treatment (with or without restricted foraging); therefore, we tested the effects of restricted foraging using only adults reared without drumming in 2014. Data were pooled across colonies.

Behavioural data were broken down as follows: observed on or off the nest, active (while on the nest), and nest abandonment. We calculated the proportion of instances (out of a maximum of 6 observations per day) that an individual was observed on the nest during the scanning period, and of those, the proportion of instances in which it engaged in a social activity (tending or checking brood, interacting with nestmates, or constructing nest cells). To compare the effects of artificial drumming or restricted foraging on the proportion of times in a day an individual was observed on its nest, we averaged data from individuals checked on at least five separate days. To determine the effects of artificial drumming or restricted foraging on the proportion of times while observed on the nest that an individual engaged in worker-like behaviour, we averaged the social activity data from individuals that were observed on the nest for at least four days. For both analyses, we used the non-parametric Wilcoxon Ranked Sum Test, in lieu of a T-test.

Late in the colony season, adult females gradually disappear – either due to death or in preparation to over-winter in a state of diapause (a gyne-like behaviour). Therefore, we explored whether drumming or foraging restriction during larval development influenced whether an individual abandoned the nest within the first five days after emerging. We used a Chi-square test to determine whether drumming (2013) or restricted foraging (2014) affected the probability of nest abandonment.

We found no evidence that antennal drumming or restricted colony foraging during larval development influenced the behaviour of those individuals as adults (proportion instances observed on nest (Wilcoxon Ranked Sum Tests): drumming: $Z = 0.03$, $P = 0.98$, restricted foraging: $Z = -0.10$, $P = 0.92$; proportion instances on nest engaged in social activity (Wilcoxon Ranked Sum Tests): drumming: $Z = -0.05$, $P = 0.96$; restricted foraging: $Z = 0.00$, $P = 1.00$; probability of nest abandonment (Fisher’s Exact Test): drumming: $P = 0.19$; restricted foraging: $P = 0.72$; see Table below for descriptive statistics). Low sample size precluded behavioural analyses to determine the extent to which nutrition and drumming interacted to influence adult behaviour.

We expected *P. fuscatus* individuals reared under nutritional stress would be more likely to engage in foraging, as was found for the related *P. metricus* in a lab-based study (1), or other nest work. We suspect the lack of behavioural effects may be due to a lack of brood in the nest (typical of late season colonies), and thus a lack of stimuli for the performance of worker tasks. Moreover, unlike studies conducted on colonies in the lab (1, 2), field-reared adult wasps often disappear from their natal nests late in the season, and their behaviour off the nest (e.g., sugar foraging or clustering

behaviour) cannot be tracked. Additional work will be necessary to better address the effects of maternal factors on adult *Polistes* behavior.

Table. Effects of simulated drumming and restricted foraging on behaviour. n = number of individuals. Data were pooled across colonies.

Observed on nest = proportion of instances (out of 6) within a scan bout that an individual (checked for at least 5 days) was observed on the nest.

Social activity = proportion of instances that an individual (observed on the nest on at least 4 separate days) was engaged in a social activity when observed on the nest.

Nest abandonment = number of individuals that were no longer observed on or near the nest within 5 days after emerging as an adult

	2013		Treatment	
	No drumming	Drumming	No Drumming Unrestricted foraging	2014 Restricted foraging
Observed on nest	0.75 ± 0.07 (20)	0.76 ± 0.12 (7)	0.38 ± 0.10 (7)	0.33 ± 0.07 (4)
Social activity	0.09 ± 0.02 (15)	0.12 ± 0.06 (4)	0.16 ± 0.13 (3)	0.06 ± 0.02 (2)
Nest abandonment	5 of 20	0 of 7	2 of 7	1 of 4

References

1. Daugherty THF, Toth AL, Robinson GE. Nutrition and division of labor: Effects on foraging and brain gene expression in the paper wasp *Polistes metricus*. *Molecular Ecology*. 2011;20(24):5337-47.
2. Jandt JM, Thomson JL, Geffre AC, Toth AL. Lab rearing environment perturbs social traits: a case study with *Polistes* wasps. *Behavioral Ecology*. 2015;26(5):1274-84.